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Environmentally Responsive Cationic Liposome-DNA Complexes for Cell Delivery

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Cationic lipids continue to attract attention as synthetic nucleic acid (NA) vectors, and are broadly used for gene transfection (with plasmid DNA) and silencing (with siRNA) including applications in clinical trials. This study elucidates the superior behavior of environmentally responsive multivalent cationic lipids containing degradable disulfide bond (labeled CMVLs). Remarkably, characterization of the complexes prepared with CMVLs containing degradable disulfide bond (compared to the non-degradable analogs) reveals the significant effects of stability control. The characterization of CMVL-NA complexes was assessed using varied CMVL/NA charge ratios in combination with different ratios of CMVLs to neutral lipids DOPC and Cholesterol. Small angle X-ray scattering (SAXS) was used to probe the initial formation of the lamellar structure of (L_α^c) CMVL-DNA complexes and their instability in different reducing environments. The X-ray scattering demonstrates the disassembly of CMVLs-NA complexes in a reducing environment that mimics the cytoplasmic milieu. SAXS under reducing conditions reveals DNA bundle formation by the cleaved headgroup of CMVLs with highly charged headgroups (CMVL5, n=5+) but not for smaller headgroup charge (CMVL2, n=2+). In comparison to non-degradable analogs, CMVL-NA complexes exhibit an unexpectedly large reduction in cytotoxicity while retaining high transfection efficiency (Shirazi et al., *Biochim. Biophys. Acta - Biomembranes* **2011,** 1808, 2156–2166). The current results clearly demonstrate CMVLs as suitable building blocks to form stable assemblies in non-reducing environment with triggered disassembly induced by a switch to reducing environments. Funded by NIH GM-59288, DOE BES DE-FG-02-06ER46314, and NSF-DMR 1101900, C. Leal was partially funded by the Swedish Research Council(VR).

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In Silico and in Ex-Vivo Experiments Indicate the Potential of Nanoparticles Composed of RNA-Bolaamphiphile Complexes as a Therapeutic siRNA Delivery Vehicle

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Speciific siRNA that are designed to silence oncogenic pathways can be used for cancer therapy.

However, naked siRNAs have short half-lives in the blood stream and have difficulties in crossing biological membranes due to their negative charges. These biological barriers can be overcome by using siRNA-bolaamphiphile complexes. Bolaamphiphiles have two positively charged hydrophilic head groups connected by a hydrophobic chain and they have a relatively low toxicity and long persistence in the blood stream. In this study, we investigated the interactions between bolaamphiphiles and siRNA and correlated these interactions with gene silencing efficacy. Two different types of bolaamphiphiles have been studied, GLH19 and GLH20, each having different head group structures. Our explicit solvent molecular dynamics (MD) simulations showed that these bolaamphiphiles associate with RNA due to (1) electrostatic interactions, (2) hydrogen bond interactions and (3) hydrophobic interactions. We found that GLH20 head groups interacted with the RNA by electrostatic and strong hydrogen bond interactions, while GLH19 head groups associated with the RNA mainly by electrostatic interactions. Studies of agarose gel electrophoresis indicated weaker interaction of GLH19 with siRNA as compared to the interaction between siRNA and GLH20. In high salt concentration and in the presence of Triton X100, siRNA was released from GLH19 complexes, while the siRNA-GLH20 complex remained intact. In transfection experiments, the siRNA-GLH19 complex was more effective than siRNA-GLH20 complex. These MD simulations and experimental results suggest that siRNA is released from the siRNA-GLH19 complex within the cells faster than from the siRNA-GLH20 complex, enabling more effective gene silencing when the siRNA-GLH19 complex is used for the transfection**.** Therefore,we propose that siRNA-GLH19 complexes are strong candidates as vehicles for therapeutic purposes.

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Small Interfering RNA Transfection Barriers Across a Lipid Membrane

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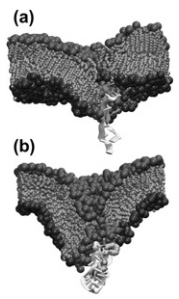
Small interfering RNA (siRNA) molecules play a pivotal role in silencing gene expression via the RNA interference (RNAi) mechanism. We examine the effect of a bare siRNA on the structure and mechanical properties of a dipalmitoylphosphatidycholine (DPPC) bilayer. Our investigations are based on all-atom molecular dynamics (MD) simulations reaching 0.5 microseconds for systems containing 733,000 atoms. These simulations reveal that the bare siRNA transforms the liquid disordered (L_α) phase of the lipid bilayer at temperature T = 323 K into a phase of frustrated lipid gel domains which consist of lipid molecules with interdigitated and non-interdigitated hydrocarbon chains. We have also performed all-atom MD simulations to examine the interaction between the DPPC bilayer and a complex consisting of a siRNA with an oleic acid (OA) molecule connected to each 3' end. In this case, the bilayer transforms into the liquid-ordered(L_o) phase. Steered MD simulations are also performed to study the transfection of a bare siRNA and siRNA/OA complex across the DPPC bilayer. Results for the transfection force and stress profiles across the lipid membrane will be presented.



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Interactions of Gold Nanoparticles with DNA: Interplay of Sequence, Ligands Charge and Polarity

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DNA template can trigger the self-assembly of metal or semiconductor nanoparticles into programmable molecular architectures. The performance of DNA-nanoparticle system strongly depends on the size and ligand chemistry of nanoparticles. We performed molecular dynamics simulations to investigate the effect of colloidal gold nanoparticle (GNP) ligands charge and polarity on the ability to bind DNA molecules. We tailored the surface of GNP by introducing different terminal functionality to thiolated ligands, such as hydrophobic, polar and charged groups. We found that uncharged GNPs and GNPs with cationic ligand charge density of less than 10% can only bind to the minor groove of DNA. Whereas GNPs with ligands charge density of higher than 10% can bind to major or minor groove. Binding to major groove result in significant distortion and wrapping of DNA around the GNP. The distortions of the DNA helical structure strongly depends on the ligand charge density. Also at higher nanoparticle concentration and low charge densities, the ligand hydrophobicity can disrupt the hydrogen bonding between base pairs of DNA strands and leads to unwinding of DNA helix. We observed that by tuning the cationic charge density and polarity of GNP we can control the binding modes and DNA structural modifications.

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Design and Assembly of an Artificial Oxygen-Evolving Complex in DNA Nanostructures

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The need for a renewable and sustainable energy source has become apparent, and focusing on a bioinspired approach emphasizing the water splitting mechanism in photosynthesis is a challenging, yet practical and attainable strategy. In this work, a stable framework consisting of a three-dimensional DNA tetrahedron has been used for the design of a biomimic of the Oxygen-Evolving Complex (OEC) found in natural Photosystem II (PSII). In nature, one of Photosystem II's core proteins, D1, is degraded every half hour in the presence of sunlight. D1's sensitivity photodamage resides in triplet state formation of chlorophyll $P680^+$, the primary donor of PSII. Our project aims to build the heart of the OEC, including the Mn_4CaCl metal cluster and its protein environment in stable DNA nanocages, which can be connected to a photostable artificial reaction center that performs light-induced charge separation. The peptide sequences responsible for coordinating the cluster have been identified through x-ray structure analysis. RTruncated regions of the peptide sequences containing Mn_4CaCl ligation sites are implemented in the design of the aOEC and are attached to sites within the tetrahedron to facilitate assembly. Crystals of the